COMPLIANCE GUIDELINES TO CONTROL
LISTERIA MONOCYTOGENES IN POST-LETHALITY EXPOSED
READY-TO-EAT MEAT AND POULTRY PRODUCTS

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A. Requirements of the Final Rule

Listeria monocytogenes is a pathogen that is widely distributed in the environment such as plant, soil, animal, water, dirt, dust, and silage. Because L. monocytogenes can be found in slaughter animals and in raw meat and poultry and other ingredients, it can be continuously introduced in the processing environment. The pathogen can cross-contaminate food contact surfaces, equipment, floors, drains, standing water and employees. In addition, the pathogen can grow in damp environments and can establish a niche and form biofilms in the processing environment that is difficult to eliminate during cleaning and sanitizing. Other characteristics of L. monocytogenes that makes it a formidable pathogen to control are its heat and salt tolerance and its ability to grow at refrigeration temperatures.

The lethality treatment received by processed ready-to-eat (RTE) meat and poultry products eliminate the pathogen, however products can be re-contaminated by exposure after the lethality treatment during peeling, slicing, repackaging, and other procedures. Several foodborne illnesses resulting in hospitalization, miscarriage and death have been linked to the consumption of deli meats and hotdogs containing L. monocytogenes. The cause of L. monocytogenes contamination in these outbreaks was traced to post-lethality exposure and contamination by the pathogen. Hot dogs and deli meats are examples of RTE meat and poultry products that receive a lethality treatment to eliminate pathogens, and are subsequently exposed to the environment during peeling, slicing, and repackaging operations. If L. monocytogenes is present in the equipment used for peeling, slicing or repackaging, the pathogen can be transferred to the product upon contact. Since RTE products are consumed without further cooking for safety, there is a possibility of the occurrence of foodborne illness.

RTE meat and poultry processing plants must include control programs for Listeria monocytogenes in their HACCP plan, Sanitation SOP or prerequisite programs to prevent its growth and proliferation in the plant environment and equipment, and cross-contamination of RTE products. The final rule for the control of Listeria monocytogenes include three alternative methods that establishments can use in the processing of RTE meat and poultry products during post-lethality exposure. These alternatives are based on different ways of controlling L. monocytogenes used in the processing of RTE products that are exposed to the environment after the lethality treatment. The risk for contamination by the pathogen increases from alternative 1 to 3, based on the control methods used by the establishment. Alternative 1 requires an establishment to apply a post-lethality treatment and an antimicrobial agent or process to control L. monocytogenes. Alternative 2 requires an establishment to apply either a post-lethality treatment or an antimicrobial agent or process. In alternative 3, the establishment does not apply any post-lethality treatment or antimicrobial agent or process, so it is required to have a sanitation program that includes testing food contact surfaces and holding product when tests turn out positive. An establishment must identify to which alternative their RTE product falls into based on its control program for L. monocytogenes. An establishment must apply the control methods required for the specific alternative in its

processing so it can qualify for the alternative. Each alternative has requirements that the establishment must comply to.

The compliance guidelines aim to help the establishment in its use of control methods for L. monocytogenes. Its purpose is to show establishments what the control methods used singly or in combination can achieve to prevent or eliminate L. monocytogenes contamination in the product during post-lethality exposure. Establishments can use the guidelines to determine control methods that are best suited to their processing. Some establishments may have instituted methods which they have verified to be effective in controlling the pathogen and may not need to change their methods to follow these guidelines. However, FSIS will make a determination on the effectiveness of the controls and establishment verification testing when deciding how FSIS will conduct verification in the establishment. These guidelines will be updated as necessary to include validated and other effective procedures as they become available.

Alternative 1

Alternative 1 requires the use of post-lethality treatment (which maybe an antimicrobial agent) to reduce or eliminate L. monocytogenes and an antimicrobial agent or process to suppress or limit the growth of the pathogen. For RTE products that are cooked and then removed from their cooking bag and sliced, diced or repackaged, there is a risk of cross contamination from the equipment, conveyor belts and the environment. These products need to be aseptically processed and then repackaged under strict sanitary conditions to prevent contamination from L. monocytogenes. Post lethality treatments such as steam pasteurization, hot water pasteurization, radiant heating and high pressure processing have been developed to prevent or eliminate post-processing contamination by L. monocytogenes. RTE products where post-lethality treatments were shown by studies to be effective in reducing the level of L. monocytogenes are whole or formed ham, whole and split roast beef, turkey ham, chicken breast fillets and strips, and sliced ham, sliced turkey, and sliced roast beef.

Examples of antimicrobial agents shown to inhibit listerial growth are lactates, acetates or diacetates added in the formulation and the use of growth inhibitors in the immediate packaging material. Some growth inhibitor packaging and some levels and combinations of antimicrobial agents were shown by research studies to reduce the levels of L. monocytogenes. RTE products where studies on antimicrobial agents were shown to be effective in the control L. monocytogenes are hot dogs, bologna, cotto salami, and bratwurst.

An establishment whose product or process falls in Alternative 1 must have the post-lethality treatment that reduces or eliminates the pathogen in its HACCP plan. The post-lethality treatment must be validated according to § 417.4 as being effective in reducing or eliminating L. monocytogenes and the validation should specify the log reduction achieved by the post-lethality treatment and antimicrobial agents. The effectiveness of the post-lethality treatments and antimicrobial agents must be verified and have the verification results available to FSIS personnel upon request.

The antimicrobial agent or process that limits or suppresses L. monocytogenes must be included in the establishment's HACCP plan, or sanitation SOP, or other prerequisite program. The establishments must have documentation in its HACCP plan, Sanitation SOP or other prerequisite program to demonstrate that the antimicrobial agent or process, as used, is effective in suppressing or limiting growth of L. monocytogenes. The establishment must validate and verify the effectiveness of its antimicrobial agent or process included in its HACCP plan in accordance with § 417.4. If the antimicrobial agent or process is in the Sanitation SOP, the effectiveness of the measures must be evaluated in accordance with 416.14. If the control measures for L. monocytogenes are contained in a prerequisite program other than a Sanitation SOP, the program must ensure that the program is effective and does not cause the hazard analysis or the HACCP plan to be inadequate.

Post-lethality treatments can be applied as a pre-packaging treatment, e.g. radiant heating, or as post-packaging treatments, e.g., hot water pasteurization, steam pasteurization, and high pressure processing. Some of the studies on post-lethality treatments are reviewed in section B. Establishments should refer to the details of the studies if they want to use the intervention method in their processing. The guidelines will be updated to include studies or other methods as they become available. Studies on post-lethality treatments showed reductions of inoculated L. monocytogenes from 1 to 7 $\log_{10}$ CFU/g depending on the product type, and duration, temperature and pressure of treatment. Higher log reductions were obtained when both pre-packaging and post-packaging surface pasteurizations were applied, and when post-lethality pasteurization was combined with the use of antimicrobial agents.

An establishment can use available published research studies as reference for their validation provided it uses the product type or size, the type of equipment, time, temperature, pressure and other variables used in the study in order to result in the same level of reduction of L. monocytogenes. An establishment that uses products, treatments or variables other than those used in the studies must perform its own validation studies to determine the effective reduction of L. monocytogenes as a result of the post-lethality treatment or antimicrobial agent applied to the products. Some of the published studies use different products and report a range of levels of reduction of L. monocytogenes. In this case, the establishment must validate the use of the post-lethality treatment or antimicrobial agent for their specific products. The establishment must specify the level of reduction achieved by the post-lethality treatment or antimicrobial agent applied in their validation. Aside from validation of the post-lethality treatment and antimicrobial agent, the establishment must verify its effectiveness by testing for L. monocytogenes.

Antimicrobial agents can be added to the product formulation, to the finished product or to the packaging material to inhibit growth of L. monocytogenes in the post-lethality exposed product during its refrigerated shelf life. Studies on antimicrobials added to the packaging material or active packaging showed a 1-2 $\log_{10}$ CFU/g reduction of L. monocytogenes during the refrigerated shelf life of the products. Lactates, acetates and diacetates are some antimicrobials added to the formulation of RTE meat and poultry

products. Based on published studies, growth reduction or inhibition achieved by adding these antimicrobials to product formulation depends on a variety of factors. Depending on the amount of antimicrobials and other growth inhibitors added to the product formulation and other ingredients in the product, growth inhibition of L. monocytogenes was shown to range from 30 days to 120 days at refrigerated temperatures. Some published studies on antimicrobials are reviewed in section C. Establishments should refer to the details of the studies if they want to use the intervention method in their processing.

An antimicrobial process that controls the growth of L. monocytogenes in the post-lethality environment is a lethality process that renders a RTE product shelf stable. Shelf stable products are formulated with salt, nitrites and other additives, and processed to achieve a water activity, pH and moisture-protein ratio that will reduce the level of L. monocytogenes and other pathogens during processing. In addition, the lethality treatment exerts a continuing bactericidal and bacteriostatic effect and does not support the growth of L. monocytogenes and other pathogens during the shelf life of the product at ambient temperatures. In this case, the antimicrobial process could serve as a both a post-lethality treatment and growth inhibitor. The establishment should have documentation on file to demonstrate the effectiveness of the lethality treatment through the shelf life of the product. Examples of shelf stable RTE products are country cured ham, pepperoni, salami, and jerky.

Another antimicrobial process that controls the growth of L. monocytogenes in the post-lethality environment is freezing of RTE products. Freezing prevents the growth of any microorganisms in the product because their metabolic activities are arrested, but depending on the method and length of freezing and other factors, some microbial kill can also result. Like other microorganisms, L. monocytogenes is resistant to freezing. Once the product is thawed, metabolic activities of microorganisms may resume, depending on whether the microorganisms are killed, injured, or not affected at all. Therefore this antimicrobial process is only effective while the product is frozen. Labels of RTE frozen products contain cooking instructions for the frozen product and for thawed and refrigerated product, and instructions for thawing at refrigerated temperatures. Examples of frozen RTE products are fully cooked frozen chicken nuggets, fully cooked frozen chicken breast patties or fully cooked frozen dinners.

The establishment can include the antimicrobial treatment that limits or suppresses L. monocytogenes in the HACCP plan, or Sanitation SOP or prerequisite program. However, the establishment must show the effectiveness of the antimicrobials in suppressing or limiting L. monocytogenes in these programs. An establishment can use published studies as reference for its validation as long as it uses the same treatment variables as those used in the study. These variables include among others, specific antimicrobial agents, concentration, time and temperature of effectiveness and others. Use of antimicrobial singly or in combination, with different concentration and other variables, and for products not used in the studies must be validated or tested for their effectiveness. This must be validated for the HACCP plan, or documented in the Sanitation SOP or other prerequisite programs. The establishment must verify that the

antimicrobial program is effective by testing product for L. monocytogenes and must verify that it does not cause the hazard analysis or the HACCP plan to be inadequate.

An establishment with products in Alternative 1 must maintain sanitation in the post-lethality processing environment in accordance with part 416. The establishment must make the verification results that demonstrate the effectiveness of its controls, whether from carrying out its HACCP plan, or its Sanitation SOP, or other prerequisite program, available upon request to FSIS inspection personnel.

Establishments have been using prerequisite programs before in their processing operations, and the Agency has recently accepted the use of prerequisite programs as an option in another policy. However, giving the establishment the option to include the antimicrobial agent or process in a prerequisite program in this rule is the first time prerequisite programs are recognized in codified regulations.

An establishment with products in Alternative 1 must have a post-lethality treatment that effectively reduce or eliminate L. monocytogenes, and an antimicrobial agent or process that suppresses any growth of the pathogen and extend the effect of the post-lethality treatment during the shelf life of the product. The Agency considers these treatments to be effective in controlling the pathogen to result in a safe RTE product. If an establishment has an effective Sanitation SOP, any contamination by L. monocytogenes would be very small, so the post-lethality treatment and the antimicrobial will be able to reduce or eliminate this contamination. If there is gross contamination, the effectiveness of the treatments may be reduced or negated. Therefore the Agency is relying on the establishment's Sanitation SOP to prevent contamination with L. monocytogenes, and the post-lethality treatment and antimicrobials to further reduce or eliminate the pathogen.

Because of this combination of controls, the Agency is not requiring establishments to have a testing program for food contact surfaces. However, the establishments can test food contact surfaces for L. monocytogenes, or its indicator organisms, Listeria spp. or Listeria-like organisms, to verify that their Sanitation SOP is effective. L. monocytogenes belongs to the Listeria group or genus of microorganisms, therefore a positive test for Listeria spp. or Listeria-like organisms would indicate the potential presence of the pathogen. If these specific indicator organisms test negative, this is indicative that L. monocytogenes is not present. Aerobic plate counts (APC), total plate counts (TPC), and coliforms are not appropriate indicator tests for L. monocytogenes. Results from these tests do not indicate the presence or absence of the pathogen. Guidelines on sanitation procedures and food contact surface testing for L. monocytogenes or its indicator organisms, Listeria spp. or Listeria-like organisms, are found in section D.

Alternative 2

An establishment that identifies its products in Alternative 2 must apply either a post lethality treatment or an antimicrobial agent or process that controls the growth of L. monocytogenes. The establishment must have the post-lethality treatment in its HACCP plan and must be validated according to § 417.4 as being effective in reducing or

eliminating L. monocytogenes and should specify the log reduction achieved by the post-lethality treatment. The effectiveness of the post-lethality treatment must be verified by testing for L. monocytogenes and have the verification results available to FSIS personnel upon request. If an establishment has a product identified in Alternative 2 and uses a post lethality treatment to control L. monocytogenes in its product, it is not required to test food contact surfaces in the post-lethality environment. However, it can test food contact surfaces for L. monocytogenes, or its indicator organisms (Listeria spp. or Listeria-like organisms), or it could be subject to frequent verification testing by FSIS if it does not.

An establishment in Alternative 2 that only uses an antimicrobial agent or process to control L. monocytogenes in its product must have the agent or process included in the establishment's HACCP plan, or sanitation SOP, or other prerequisite program. The establishments should have documentation in its HACCP plan, Sanitation SOP or other prerequisite program to demonstrate that the antimicrobial agent or process, as used, is effective in suppressing or limiting growth of L. monocytogenes. The establishment should document the log levels of the pathogen that the antimicrobial agent or process can suppress and the length of time in days that the antimicrobial is effective. The establishment must validate and verify the effectiveness of its antimicrobial agent or process included in its HACCP plan in accordance with § 417.4.

If the antimicrobial agent or process is in the Sanitation SOP, the effectiveness of the measures must be evaluated in accordance with 416.14. If the control measures for L. monocytogenes are contained in a prerequisite program other than a Sanitation SOP, the program must ensure that the program is effective and does not cause the hazard analysis or the HACCP plan to be inadequate. The establishment should document its antimicrobial agent or process, its implementation and its verification results sufficiently in order to show that the HACCP plan is adequate in controlling the pathogen. The establishment must verify that the antimicrobials are effective by testing for L. monocytogenes and have the verification results whether from carrying out its HACCP plan, or its Sanitation SOP, or other prerequisite program, available upon request to FSIS inspection personnel.

If an establishment's product is in Alternative 2 and uses an antimicrobial agent or process that suppresses or limits the growth of L. monocytogenes in its product, it should maintain sanitation in the post-lethality environment in accordance with part 416. The sanitation program must include testing for food contact surfaces in the post-lethality environment to ensure that the surfaces are sanitary and free of L. monocytogenes or its indicator organisms (Listeria spp. or Listeria-like organisms). Studies on antimicrobials showed growth inhibition of L. monocytogenes if present at low levels of contamination during the shelf life of the RTE product. Antimicrobials were not shown to be effective at higher levels of contamination, so an effective sanitation program, which includes verification testing for food contact surfaces must be implemented at the same time that antimicrobials are used.

The sanitation program must provide for testing food contact surfaces in the post-lethality processing area to ensure that surfaces are sanitary and free of l. monocytogenes or its indicator organisms. It must include the frequency of testing and identify the size and location of the sample sites to be sampled. It should include an explanation of why the testing frequency is sufficient to ensure that effective control of L. monocytogenes or its indicator organisms is maintained. In addition, the establishment must identify the conditions under which the establishment will implement hold-and-test procedures following a positive test for L. monocytogenes or its indicator organisms. The product will be subject to FSIS verification testing, because the establishment is not relying on a post-lethality treatment to eliminate L. monocytogenes.

Alternative 3

A post-lethality exposed product that does not use a post-lethality treatment or an antimicrobial agent or process to control the growth of L. monocytogenes fall under Alternative 3. An establishment producing this product must control the pathogen in its post-lethality processing environment through the use of sanitation control measures. Because the establishment is not relying upon a post-lethality treatment or an antimicrobial agent or process to control L. monocytogenes, the product will be subject to frequent FSIS verification testing. Examples of products in this alternative are fully cooked meat and poultry that are packaged and refrigerated such as hotdogs, deli meats, chicken nuggets, or chicken patties.

For this alternative, the establishment must maintain sanitation in the post-lethality processing environment in accordance with part 416. The sanitation program must provide for testing food contact surfaces in the post-lethality processing area to ensure that surfaces are sanitary and free of L. monocytogenes or its indicator organisms. The testing program should include the frequency of testing, identify the size and location of the sample sites and include an explanation of why the testing frequency is sufficient to ensure that effective control of L. monocytogenes or its indicator organisms is maintained. In addition, the establishment should identify the conditions under which the establishment will implement hold-and-test procedures following a positive test for L. monocytogenes or its indicator organisms on a food contact surface.

Moreover, an establishment that produces a deli product or a hotdog product must verify that the corrective actions that it takes with respect to sanitation after an initial positive test for L. monocytogenes or its indicator organisms on a food contact surface in the post-lethality processing environment are effective. The corrective action must indicate steps that the establishment will take to clean and sanitize the suspected food contact surfaces to eliminate the contamination. The verification of the effectiveness of the corrective action can be shown by follow-up testing that includes a targeted test of the specific site on the food contact surface area that is the most likely source of contamination by the organism and other additional tests in the surrounding food contact surface area as necessary to ensure the effectiveness of the corrective actions. During this follow-up testing, if the establishment obtains a second positive test for L. monocytogenes or an indicator organism, the establishment must hold lots of product that may have become

contaminated by contact with the food contact surface until the establishment corrects the sanitation problem indicated by the test result.

Further, in order to be able to release into commerce the lots of product that may have become contaminated with L. monocytogenes from the food contact surface, the establishment must sample and test the lots for L. monocytogenes using a sampling method and frequency that will provide a level of statistical confidence that ensures that each lot is not adulterated with L. monocytogenes. If the product tests positive for L. monocytogenes, the product is considered adulterated and must be withheld from commerce. The establishment may destroy the held product , or rework the held product using a process that is destructive of L. monocytogenes. The establishment must document the results of the testing and the disposition of the product. An example of a hold-and test scenario can be found in section E-VIII.

An establishment with products in Alternative 3 is likely to be subject to more frequent verification testing by FSIS than an establishment with products in Alternative 1 or 2. This is because the products in Alternatives 1 and 2 are formulated and/or processed to reduce or eliminate L. monocytogenes or limit its growth in the RTE product and present a lower risk than products in Alternative 3 that do not have these interventions. Likewise, an establishment in Alternative 3 that produces deli meat or hotdog products is likely to be subject to more frequent verification testing than one that does not produce such products because deli and hotdog products were ranked as higher risks for L. monocytogenes contamination by the risk assessment.

Labeling

Antimicrobial agents that are added to RTE products, either to the formulation or to the finished RTE product, and those that are included in the primary packaging material of RTE products are required to be included in the ingredients statement of the product label. In addition, establishments that use a post-lethality treatment or an antimicrobial validated to effectively eliminate or reduce L. monocytogenes, or suppress or limit its growth in the product can make claims or special statements on the labels of their products regarding the presence and purpose of use of the substances. The purpose of such claims is to inform consumers about measures taken by the processor to ensure the safety of the product and enable consumers to make informed purchase decisions. Such claims are voluntary, and may be of value to consumers especially those in groups most vulnerable to foodborne illness. Processors need to document their validation of these claims. An example of a statement that can be made is: “Potassium lactate added to prevent the growth of L. monocytogenes.” All labeling claims and label changes to add such claims must be submitted for evaluation and approval to the FSIS Labeling and Consumer Protection Staff.

Estimates of Annual Production Volume

An establishment that produces post-lethality exposed RTE products shall provide FSIS with estimates of annual production volume and related information (such as the establishment’s testing program) for the types of meat and poultry products processed

under Alternatives 1, 2, or 3. The establishment needs to provide the information at least annually, or more often, as determined by the Administrator. The Agency regards production volume as a more important risk factor than establishment size and therefore needs these data so that it can target its resources on higher volume operations in its verification program. FSIS will develop sampling frequencies for the establishments and the products based on these data. The Agency will make the sampling frequency available to the establishments so that they will have an indication of how the risk of L. monocytogenes is tied to verification sampling.

The form by which to collect the data will be available to establishments in paper or electronic formats. An electronic form for this purpose will be available to the establishments at all times after the rule becomes effective.

B. Studies on Post-lethality Treatments

(Mention of trade marks or commercial names does not constitute endorsement by USDA)

I. Steam Pasteurization and Hot Water Pasteurization

Post processing contamination of RTE meat and poultry is mostly confined to the surface. Pasteurization by steam and hot water acts on the surface microbial contaminants by the action of heat. Studies on surface pasteurization using steam or hot water were shown to be effective in reducing this contamination.

Studies by Murphy et al. (2003a) showed that post-cook hot water pasteurization and steam pasteurization resulted in a 7 $\log_{10}$ reduction of L. monocytogenes in inoculated vacuum packaged fully cooked sliced chicken. The reduction was effective when single – packaged breast fillets, 227 g- package strips and 454 g-packaged strips were heat treated at 90 C in a continuous steam cooker or hot water cooker for 5, 25 and 35 minutes respectively. These investigators developed a model called ThermoPro that could predict the thermal lethality of pathogens in fully cooked meat and poultry products during post-cook in-package pasteurization (Murphy et al., 2001, 2003b, 2003c). The model was developed using L. innocua and verified for L. monocytogenes.

II. Pre-Package Pasteurization and Post-Package Surface Pasteurization

Muriana et. al., (2002) used a stainless steel water bath (similar to the Unitherm commercial Aquaflow food processor) to submerge cooked RTE deli-style whole or formed turkey, ham and roast beef, removed from their package, inoculated with L. monocytogenes and vacuum packaged. Results show a 2-4 log decrease in the levels of L. monocyogenes in inoculated products post-cooked at 195-205 F for 2-10 min.

Pre-package surface pasteurization treatment of the fully cooked meat removed from their packaging wrap and inoculated with L. monocytogenes resulted in a 1.25 to 3.5 log reduction with a treatment time of 60-120 s at 475 to 750 F air temperature (Gande and Muriana, 2003). Surface pasteurization was applied on cooked whole and split roast beef,

whole corned beef, and whole and formed ham using a radiant oven ("Infrared Grill", Unitherm FoodSystems). Pre-package pasteurization (60 sec) combined with post-package submerged water pasteurization using formed ham (60 or 90 sec), turkey bologna (45 or 60 sec), and roast beef (60 or 90 sec), resulted in a 3.2 to 3.9 log reduction for ham, 2.7-4.3 log reduction for bologna, or a 2.0-3.75 log reduction for roast beef. The level of reduction varied depending on the method of inoculation, type of product used, treatment temperature, and residence time.

III. High Hydrostatic Pressure Processing

High pressure processing (HPP) is one of the new technologies used for food processing. This technology provides a means of ensuring food safety for those products that are difficult to be heat treated due to organoleptic effects. HPP was shown to inactivate pathogens without any thermal or chemical effects and at the same time preserve the quality of the product. Raghubeer and Ting (2003) evaluated the efficacy of high hydrostatic pressure processing in inactivating L. monocytogenes in retail-packaged samples of sliced ham, turkey and roast beef obtained from a manufacturer and repackaged in 25-g portions. Results show that an inoculum of about 10^4 L. monocytogenes cocktail in these 3 products and HPP treatment at 87,000 psi for 3 minutes showed no recovery of L. monocytogenes after 61 days of storage at 34° F. There were no pressure-injured cells detected. There were no adverse organoleptic effects detected on the 3 HPP treated products during the 61-day shelf life study. No signs of spoilage were seen on all 3 products after 61 days of storage, and for 100 days for ham and turkey. According to the investigators, the normal shelf life of these products is 30 days, so the HPP treatment extended the shelf life of the products.

C. Studies On The Use of Antimicrobial Agents

I. Addition of Lactates, Acetates, Diacetates to Meat Formulations

Studies have shown that lactic acid and acetic acid have significant antimicrobial activity in broth and food systems. Sodium and potassium salts of these acids, when added to processed meat formulation are also known to potentially inhibit pathogenic bacteria especially L. monocytogenes. These antimicrobials inhibit growth of pathogens by inhibiting their metabolic activities. Interest in these antimicrobials is in the growth inhibition of L. monocytogenes in post lethality exposed RTE meat and poultry products.

FSIS recently increased the permissible levels of sodium acetate as a flavor enhancer in meat and poultry products, and of sodium diacetate as a flavor enhancer and as an inhibitor of pathogen growth to 0.25 %(65 FR 3121-3123/2000). The rule also permitted the use of sodium lactate and potassium lactate in meat and poultry products at 3 %, corresponding to a 4.8 % of the 60 % commercial product (except for infant formulas and infant food) for the purposes of inhibiting the growth of certain pathogens. The addition of antimicrobials in the formulation must be included in the ingredient statement of the label. Several studies used these antimicrobials to show their ability to inhibit growth of L. monocytogenes in different meat formulations.

Seman et al., (2002) developed a mathematical model capable of predicting the growth or stasis of L. monocytogenes in commercial cured meat products using a response surface method. The model can be used by manufacturers in the determination of the appropriate amounts of potassium lactate and sodium diacetate to be added to cured meat products that are organoleptically sensible and will not support the growth of L. monocytogenes. Thirty products were formulated by using a variety of raw material sources such as pork trimmings, trimmed turkey breast halves and four-muscle ham. Varying amounts of potassium lactate and sodium diacetate were added to the meat formulation and the meats were processed into different products. After chilling, the products were stripped of their casings, sliced into 25-g slices, placed into pouches, and inoculated with L. monocytogenes by applying to the surface of 100g of cured meat (four slices).

The results show that increasing amounts of potassium lactate syrup and sodium acetate decreased the growth rate of L. monocytogenes, while increasing finished product moisture increased the growth rate. Sodium chloride content was not significant but was found to have a negative correlation to growth rate. The investigators provided final regression equation predicting the growth of L. monocytogenes in cured RTE meat products stored at 4° C. The investigators used predictive model performance factors and a simple linear regression analysis to evaluate the model generated in this study. They verified the accuracy of the model by comparing with actual L. monocytogenes growth data from independent challenge study conducted with for four different commercial RTE meat products using similar storage conditions. Performance factors calculated and evaluated for control products (those not containing potassium lactate and sodium diacetate) indicated that on the average, the predicted growth of L. monocytogenes exceeded those of the observed values by about 24 %.

This study provided a useful model in determining the target amounts of potassium lactate and sodium acetate for cured meat product formulations to inhibit the growth of L. monocytogenes. The calculations would also require knowledge of the finished product sodium chloride and moisture contents. The investigators advised that this validated model is specific to the products designed for the study and the L. monocytogenes strains used. Testing of this model in other environments and with other Listeria spp., and to formulations that are outside the model's limits may result in different maximum growth rates. This study was used as the basis for the Opti.Form Listeria Control Model.

The Opti.Form Listeria Control Model (PURAC) is a unique tool to calculate the levels of lactate and diacetate required to retard the growth of Listeria monocytogenes in cured meat and poultry products. The model is based on the study detailed in the paper by Seman et al, 2002, above. The model, which is available on CD-Rom includes:

- instructions on how to use the model
- explanation on the development of the model
- information on the anti-microbial effect of lactate and diacetate
- lactates and diacetates and use of these products
- regulations and labeling
- literature references

To receive a free copy of the model on CD-Rom, call: 888-899 8229, E-mail pam@purac.com

Bedie et al., (2001) evaluated the use of antimicrobials, included in frankfurter formulations, on L. monocytogenes populations during refrigerated storage. Fully cooked and cooled frankfurters were inoculated with 10^3 to 10^4 CFU /cm^2 of L. monocytogenes after peeling and before vacuum packaging. Samples were stored at 4° C for up to 120 days and sampled for testing on assigned days. Results are as follows:

ANTIMICROBIAL	LEVEL (%)	L. MONOCYTOGENES GROWTH INHIBITION
Sodium lactate	3	70 days no pathogen growth
Sodium diacetate	0.25	50 days no pathogen growth
Sodium acetate	0.25, 0.50	20 days no pathogen growth
Sodium lactate	6	120 days no growth and reduced pathogen growth
Sodium diacetate	0.5	120 days no growth and reduced pathogen growth
Control	0.0	Increased to 6 logs in 20 days

No pathogen growth refers to no increase in the number of inoculated L.monocytogenes cells (bacteriostatic); while reduced pathogen growth refers to a decrease in the number of inoculated L. monocytogenes cells (bactericidal) in the product. In this study, tables showed the reduction varied with storage days, but was up to 1.0 log on some days. Antimicrobials were found to have no effect on pH except for sodium diacetate at 0.5 % which reduced the initial pH. Using the formulations and conditions in the study, establishments can add 3 % sodium lactate in the frankfurter formulation and obtain no growth of L. monocytogenes up to 70 days at refrigerated storage of 4° C. If the lethality treatment is adequate to eliminate L. monocytogenes, then the only probable source of L. monocytogenes would be from exposure of the product during peeling and repackaging. However, the establishment's sanitation program may keep the numbers to a very low level, and 3 % sodium lactate included in the formulation would inhibit the growth of L. monocytogenes during the product's refrigerated shelf life. Levels of sodium lactate at 6.0 % and sodium diacetate at 0.5 % showed a reduction of the pathogens, however these levels are above the permitted levels.

This study by Samelis et al., (2002) used similar treatments, processing and inoculation procedures and frankfurter formulations as the previous study described above. However, in this study combinations of antimicrobials were used, and in combination with hot water treatment. Hot water treatment involved immersion of frankfurters, with two product links in a package to 75 or 80° C for 60 s. Storage at 4° C shows:

TREATMENT	LEVELS (%)	L. MONOCYTOGENES GROWTH INHIBITION
Sodium lactate	1.8	35-50 days no growth
Sodium lactate + sodium acetate	1.8 0.25	120 days no growth; 35-50 days growth reduction
Sodium lactate + Sodium diacetate	1.8 0.25	120 days no growth; 35-50 days growth reduction
Sodium lactate + Glucuno-delta-lactone	1.8 0.25	120 days no growth, 35-50 days growth reduction
Hot water treatment (80° C, 60 s) + Sodium lactate	1.8	Inoc. population reduced by 0.4-0.9 log CFU/cm^2 , and 50-70 days growth reduction by 1.1-1.4 CFU/ cm^2
Hot water treatment (80° C, 60 s)		Increase in growth to about 6-8 logs in 50 days
Control, no treatment		Increase in growth to about 6 logs in 20 days and 8 logs thereafter up to 120 days

*3 % of a 60 % (wt/wt) commercial solution.

Glass et. al., (2002) evaluated sodium lactate and sodium diacetate on wieners and cooked bratwurst containing both beef and pork supplied by a commercial manufacturer. Antimicrobial solutions used were sodium lactate and sodium diacetate singly or in combination at varying concentration. Wieners were repackaged in gas-impermeable pouches, then surface-inoculated with L. monocytogenes mixture on multiple areas of the surface of each link. Packages were vacuum-sealed and stored at 4.5º C for up to 60 days. Two types of cooked bratwurst from a commercial manufacturer were evaluated: bratwurst that was cured and naturally smoked and bratwurst that was uncured and unsmoked. Bratwurst was stored at 3 or 7° C for up to 84 days.

The surface treatment consisting of dipping wieners into solutions containing up to 6 % lactate and up to 3 % diacetate for 5 s did not delay pathogen growth, indicating that dipping wieners in the lactate/diacetate solutions is not an efficient way to apply the antimicrobials . However, the inclusion of lactates and diacetates in the formulation was found effective in inhibiting growth of L. monocytogenes. Results are as follows:

PRODUCT	Sodium Lactate (%)	Sodium diacetate (%)	L. monocytogenes levels (CFU/pkg)
Bratwurst uncured, unsmoked	3.4	0.1	Growth delayed for 4-12 weeks at 7 and 3° C storage, respectively.
	2.0	0.0	Growth delayed for 1-2 weeks at 7 and 3° C storage, respectively.
Bratwurst cured, smoked	3.4	0.1	Growth inhibited for 12 weeks at 7 and 3° C storage.
	0.0	0.0	Growth up to 1 log after 4 weeks at 7 and 3° C
Wieners	3.0	0.0	Growth inhibited for 60 days at 4.5° C
	1.0	0.1	Growth inhibited for 60 days at 4.5° C

Study by (Porto et al., 2002) used freshly processed peeled frankfurters in vacuum sealed packages obtained from a commercial manufacturer. Two formulations of links were used in the study: one with added 2 or 3 % potassium lactate and the other without added potassium lactate. Frankfurters were aseptically removed from their original package, repackaged, and inoculated with a mixture of L. monocytogenes. The packages were vacuum-sealed to 95 kPa and incubated at 4 and 10° C.

Results show that addition of 2 % or 3 % potassium lactate in frankfurters can appreciably enhance safety by inhibiting or delaying the growth of L. monocytogenes during storage at refrigeration or abused temperatures. The viability of the pathogen was influenced by pH, and the levels of lactate added, but not by the presence of indigenous lactic acid bacteria.

Potassium lactate (%)	Inoculum CFU/pkg	Storage temp °C)	Days Storage	L. monocytogenes levels (CFU/package)
2.0	20	4	90	Remained at about 1.6 log
3.0	20	4	90	Remained at about 1.4 log
3.0	500	4	90	Remained at about 2.4 log
0.0	20	4	90	Increased to about 4.6 log
0.0	500	4	90	Increased to about 5.0 log
2.0	20	10	60	Remained at about 1.4 log
3.0	20	10	60	Remained at about 1.1 log
0.0	20	10	60	Increased to about 6.5 after 28 days, declined to about 5.0 after 60 days
3.0	500	10	60	Remained at about 2.4
0.0	500	20	60	Increased to about 6.6 log after 40 days and declined to about 5.5 log after 60 days

II. Growth Inhibitor Packaging

Growth inhibitor packaging is an intervention, which delivers an active antibacterial agent to the surface of an encased sausage product. By incorporating this special coating onto the internal surface of cellulose casings, the antilisterial treatment is transferred to the surface of the processed meat/sausage during thermal processing. Upon removal of the casing, the treatment remains active on the meat surface, providing effective protection against inadvertent Listeria contamination during subsequent peeling, collating, and packaging processes. Growth inhibitor packaging used in conjunction with functional HACCP and Good Manufacturing Practices provides the industry with one more tool in their intervention strategy to control the risk of pathogen contamination in ready-to-eat meat and poultry products.

Studies on meat formulations for hot dogs using NOJAX® AL™ (Viskase) showed that use of the casings provide a lethality hurdle to the growth of Listeria monocytogenes, not just an inhibitory effect. The lethality impact is delivered within the first hours/days of the sausage/hot dog package life. This impact is dependent on many variables but is generally in the range of 1 – 2 log kill of L. monocytogenes at high levels of inoculation. This performance has been observed in challenge studies conducted on hot dogs drawn from commercial full-scale trials at a number of commercial processing plants. In high inoculation trials, NOJAX AL has been combined with conventional growth inhibiting additives, and as expected, the lethality impact is obtained and then maintained throughout the product life cycle. In these same trials, without growth inhibiting additives, this casing produces lethality but in several weeks the remaining L. monocytogenes begin to grow.

NOJAX AL is available in the U.S. having approval by both FDA and USDA for its key component, nisin. This GRAS component must be included in the ingredient statement via a label change request to the FSIS Labeling and Consumer Protection Staff. Because this is a naturally derived polypeptide, there are storage and use-by criteria that will have to be adhered to by the user for maximum benefit. Casing shelf-life is about 60-90days with a not to exceed 85º F.

This technology can be applied to most hot dogs and sausages that are encased in cellulosic casing. This casing intervention can be used in any instance were casing is used as a mold for processed meat and poultry during thermal processing. This would include cellulose, plastic, and possibly natural casing. As part of a manufacturer's decision to use this technology, benefits are: 1) no capital costs or new equipment; 2) no change in processing steps, plant reconfigurations or introduction of process bottlenecks—essentially processor transparent in all aspects of use except casing storage requirements; 3) no impact on flavor, texture, or package appearance, and 4) minor labeling change to ingredient statement.

Since this is a surface treatment, cost will be proportional to the surface to volume ratio of the product: the larger the sausage diameter, the lower the cost per pound. In general, economic analyses put the cost of this lethality intervention at about 2-3 cents per pound of finished product, with a mid-range target price of 2.5 cents per pound for a traditional 10-to-the-pound retail pack of hot dogs.

Janes et al., (2002) investigated the effect of nisin added to zein film coatings (Z) coated onto cooked ready-to-eat chicken against L. monocytogenes. Cooked chicken samples inoculated with L. monocytogenes were dipped into Z dissolved in propylene glycol or ethanol, with or without added nisin (1,000 IU/g) and/or 1 % calcium propionate and stored at 4 C or 8 C for 24 days. After 16 d at 4 C, L. monocytogenes was suppressed by 4.5 to 5 log CFU/g with zein film coatings with nisin. The most effective treatment in the study for controlling L. monocytogenes on the surface of ready-to-eat chicken was using edible zein film coatings containing nisin at a storage temperature of 4°C.

The use of film coatings in a processing plant would be to fully process the meat products then coat them with the films. Coating can be done by spraying or dipping the processed meat products and then allowing them to dry. Zein coatings on the meat products can be dried by circulating air around the meat product using a fan. Finally, the dried coated meat products can be packaged with the usual plastic film material and refrigerated. Nisin is presently not approved in the USA for use on ready-to-eat meat and poultry products, and this study has not been tested in commercial poultry processing conditions.

Some general observations from the published studies on antimicrobials:

- Lactates, acetates and diacetates were found more effective in inhibiting growth of L. monocytogenes when used in combination than when used singly.

- These antimicrobials were found more effective when used to the maximum allowable concentration. However, higher concentrations of antimicrobials used in the formulation may affect the sensory qualities of the product, such as flavor and texture, which would necessitate sensory evaluation of treated products.

- When used in combination, the amount needed to inhibit growth may be reduced.

- These antimicrobials were found to have listeriostatic activity more than listericidal activity, i.e. they prevent growth of the pathogen more than reduce the number of cells of the pathogen, and therefore may not be effective against gross contamination of a product. The establishment's sanitation program should control gross contamination of the processing environment and equipment. Addition of antimicrobials would be effective only as part of the overall HACCP strategy.

- Including these antimicrobials in the formulation was found to be more effective in inhibiting listerial growth than dipping products in solutions of antimicrobials.

- The antimicrobial activity of lactates and acetates when used singly or in combination is affected by the level of contamination of the meat product surface, and processing factors such as pH, moisture, water activity, fat, nitrite, salt content, time and temperature of storage, and packaging atmosphere.

- Application of the treatments used in these studies is limited to the formulations, products and treatments used in the studies. Applying these studies to other products and formulations may result in different rates of growth inhibition. Therefore the effectiveness of the antimicrobials used in these studies must be verified by the establishment for other processed meat products and other storage temperatures.

- Antimicrobials used in the formulation must have an effective antilisterial activity throughout the commercial shelf life of the product. Currently the targeted commercial shelf life of refrigerated cooked meat products in the U.S.A. is 75 to 90 days.

- Using post-packaging thermal treatments in addition to antimicrobials was found to increase the total antilisterial effects of the antimicrobials.

- These antimicrobials were found to be more effective in smoked products formulated with sodium nitrite, or in products stored at strict refrigeration temperatures.

- Use of these antimicrobials may be a cost effective antilisterial method that very small establishments can use.

D. Sanitation Guidelines for Listeria monocytogenes

Control of L. monocytogenes is a challenge to a processing plant's sanitation program. The pathogen can grow in a damp environment, attach to surfaces that come into contact with raw or finished product, establish a niche and form biofilms. The sanitation program should include cleaning and sanitizing procedures that have been proven effective for the particular operation, separation of raw and RTE processing areas, traffic control, employee hygiene, and equipment flow and design among others.

Proper and effective sanitation involves both cleaning and verifying that the cleaning and sanitizing was effective. This involves developing and implementing written sanitation standard operating procedures (Sanitation SOP's). Sanitation SOP's could be viewed as the first step to designing a total system, including the HACCP plan, that will prevent, eliminate, or reduce the likelihood of pathogenic bacteria from entering and harboring in the plant environment. The Sanitation SOP's as described in 9 CFR 416.12 through 416.16, give detailed mandatory requirements for developing and implementing the sanitation program, while 416.17 describes how FSIS will verify that each establishment is meeting the Sanitation SOP regulations. In brief, the regulations require the following:

- **Development of Sanitation SOP's (416.12)** – Each establishment shall develop a written Sanitation SOP that describes all sanitation procedures to be performed each day, before and during operations with specific frequencies of each procedure and the responsible person for each task. It must also describe the cleaning process for all food contact surfaces, utensils, and equipment used to process your product(s). This document must be signed and dated by either the person responsible for the overall sanitation operations or a higher level employee in the establishment once it is implemented, and when any changes are made to the Sanitation SOP's.

- **Implementation of SOP's (416.13)** – All preoperational procedures identified in the Sanitation SOP shall be done daily, before processing operations start. Each procedure must be performed at the specified frequency and they must be monitored daily.

- **Maintenance of Sanitation SOP's (416.14)** – Each establishment shall routinely determine if the written Sanitation SOP is still effective in preventing direct product contamination and adulteration. If the Sanitation SOP is determined not to be effective because of changes in equipment, utensils, facility, operations, or personnel, changes in the procedures must be made to reflect changes.

- **Corrective Action (416.15)** – The appropriate corrective action(s) shall be taken when it has been determined by FSIS or by an establishment employee that the written Sanitation SOP has failed to prevent direct product contamination or adulteration of your product(s).

- **Recordkeeping Requirements (416.16)** – Daily records shall be maintained that describe how the sanitation activities were implemented and monitored, and all corrective actions; these records must be initialed and dated. Both computer records and paper records are appropriate however; additional controls may be needed to ensure the integrity of the electronic data.

- **Agency Verification (416.17)** – FSIS will verify the effectiveness and adequacy of the written Sanitation SOP's to ensure that they meet all of the regulatory requirements. This will be done by reviewing all records, direct observations, and microbial testing as deemed necessary.

I. General Procedures

An example of equipment and processing room cleaning using eight steps is outlined below. Cleaning should be increased and intensified during periods of construction.

1. Remove waste material. Dry clean equipment, conveyor belts, tables, floors to remove meat particles and other solid debris. Some equipment such as slicers and dicers need to be disassembled so that parts can be cleaned thoroughly.

Equipment may need to be cleaned and sanitized again after re-assembly.

2. Wash and rinse floor.

3. Pre-rinse equipment (rinse in same direction as product flow). Pre-rinse with warm or cold water – less than 140°F (hot water may coagulate proteins or "set soils").

4. Clean and scrub equipment. Always at least use the minimum contact time for the detergent/foam. Written instructions should be provided on the location of possible niches and the cleaning method to use. CAUTION: Live steam for cleaning is not acceptable.

5. Rinse equipment (rinse in same direction as product flow).

6. Visually inspect equipment (repeat steps 3 and 4 if not clean visually or by testing such as ATP bioluminescence).

7. Sanitize floor and then equipment to avoid contaminating equipment with aerosols from floor cleaning. Care should be taken in using high pressure hoses in cleaning the floor so that water won't splash on the already cleaned equipment. Hot water, at least 180°F, for about 10 seconds to sanitize equipment. Sanitizers (e.g., chlorine, quaternary ammonia, etc.) may be more effective than steam for L. monocytogenes control. If steam heating equipment in an oven or tarp, the target internal temperature is 160° F and hold for 20-30 min. Portable high-pressure, low volume cleaning equipment (131°F (55°C) with 20-85 kg/cm^2 pressure and 6-16 liters/minute) can be used.

8. Remove excess moisture. This can be done most safely and efficiently by drying. Reduced relative humidity can speed the process. Avoid any possible cross-contamination from aerosol or splash if a method other than air drying is used. If cross-contamination is suspected, repeat steps 4 – 7.

II. Determining the Effectiveness of Sanitation Standard Operating Procedures (Sanitation SOPs)

The establishment should determine if the cleaning and sanitizing procedures used was effective by visual examination or testing or both.

1) Visual inspection of the equipment and environment. Visual inspection is the minimum means of determining the effectiveness of the sanitation standard operating procedures (SOPs). It can only detect observable contamination.

a. Visually verify that no meat or product residue is on the equipment, especially those product contact surfaces and areas that may serve as niches for bacteria,

before the start of operation.

b. Record the results of the visual inspection.

c. If any residue is noted, corrective action should be taken and recorded.

d. The monitoring record should be designed to show any trends of insanitary conditions. For example, if corrective action had to be taken on the first two days of operation for more than a week, this indicates a possible problem with cleaning and would have to be investigated to determine the source of the problem (e.g., improperly trained crew on those days, types of products processed).

e. Visually verify that no meat or product residue is on the equipment, especially those product contact surfaces and areas that may serve as niches for bacteria, after post-processing cleanup.

2) Visual inspection and use of ATP bioluminescence testing. Visual verification combined with ATP testing can determine both observable contamination and contamination from bacteria and meat/poultry residues that may not be visually detectable. The combined methods are more effective in determining the effectiveness of the sanitation SOP.

a. The ATP test indicates the presence of both bacteria and meat or poultry residues and can be used to verify that no meat or poultry residue is on the equipment, esp. those product contact surfaces and areas that may serve as niches for bacteria, before the start of operation.

b. Record the results of the ATP test and visual inspection.

c. If any residue is noted or observed visually or the ATP test indicates an insanitary condition, corrective action should be taken and recorded.

d. The monitoring record should be designed to show any trends of insanitary conditions. For example, if corrective action had to be taken on the first two days of operation for more than a week, this indicates a possible problem with cleaning and would have to be investigated to determine the source of the problem (e.g., improperly trained crew on those days, types of products processed).

e. By ATP testing and visual examination, verify that no meat or product residue is on the equipment, esp. those product contact surfaces and areas that may serve as niches for bacteria, at the end of the shift.

3) Visual inspection and total plate counts (TPC). Visual verification combined with TPC can determine both observable contamination and the level of bacterial contamination. Since TPC results cannot be obtained at the time of inspection, its value is the measurement of the level of contamination. The level of contamination may assist the

establishment in determining the source of contamination and the effectiveness of the sanitation SOP.

a. Visually verify that no meat or product residue is on the equipment, esp. those product contact surfaces and areas that may serve as niches for bacteria, before the start of operation.

b. Use swabs or RODAC plates for sampling food contact surfaces, non-food contact surfaces, and the processing environment.

c. Record the results of the visual inspection.

d. If any residue is noted, corrective action should be taken and recorded.

e. Record the TPC when analysis complete.

f. The monitoring record should be designed to show any trends of insanitary conditions as determined by visual inspection or TPC. For example, if corrective action had to be taken on the first two days of operation for more than a week, this indicates a possible problem with cleaning and would have to be investigated to determine the source of the problem (e.g., improperly trained crew on those days, types of products processed).

g. Visually verify that no meat or product residue is on the equipment, especially those product contact surfaces and areas that may serve as niches for bacteria, again after post-processing cleanup.

III. Traffic Control

Controlling the movement of personnel and raw and finished products will help prevent cross-contamination of finished products by raw materials and personnel. The following are steps that should be taken for traffic control:

1. Establish traffic patterns to eliminate movement of personnel, meat containers, meat, ingredients, pallets and refuse containers between raw and finished product areas.

2. Control traffic into and within the RTE areas

   a. If possible, use air locks between raw and RTE areas.

   b. Clean dry floors are preferable to foot baths.

   c. If foot baths are used:

      i) Wear rubber or other non-porous boots.

ii) Maintain them properly.

iii) Solutions should contain stronger concentrations of sanitizer than normally used on equipment.

(1) For example, 200 ppm iodophor, 400-800 ppm quaternary ammonia compound).

(2) CAUTION: Chlorine is not recommended as it is too quickly inactivated esp. if cleated boots are used. Monitor and maintain its strength if used.

iv) Use a minimum depth of 2 inches.

d. Foam disinfectant spray on floor as people or rolling stock enter the room.

3. Employees should not work in both raw and RTE areas, if possible. If they must work in both areas, they must change outer and other soiled clothing, wash and sanitize hands, and clean and sanitize footwear.

a. Use different color smocks or helmets for raw and RTE areas so the workers and garments in the raw and RTE areas are readily distinguishable.

b. Remove outer garments (e.g., smocks) when leaving RTE areas.

4. Do not allow employees who clean utensils and equipment for raw materials to clean RTE utensils and equipment, if possible. If not possible, there should be a time separation when utensils for raw processing/handling are cleaned after RTE. The tools to clean utensils and equipment for raw materials must be different than those used to clean RTE utensils and equipment.

5. Do not permit maintenance employees in RTE areas during operations if possible. If not possible:

a. Consider the need to cease operations until a full cleaning and sanitizing is done, or,

b. Maintenance personnel must change outer clothing and any other soiled clothing, use separate tools for raw and RTE areas (or wash and sanitize tools and hands prior to entering RTE areas) and wear only freshly cleaned/sanitized footwear in such areas.

6. Use separate equipment, maintenance tools and utensils for the RTE and raw areas. If not possible, there should be a time separation between raw processing/handling and RTE processing.

7. Pallets can serve as a source of cross-contamination – pallets for raw materials should not be used in RTE areas or used for finished product.

8. Drains from the “dirty” or “raw” side should not be connected to those on the “clean” or “cooked” side.

IV. Employee Hygiene

Employee hygiene is the responsibility of both the individual and management. The employee is responsible for preventing contamination of food products and the management is responsible for ensuring the employee is properly trained and maintains good practices.

Employee responsibilities and actions should include:

1. Use a 20 second hand wash after using restroom facilities.

2. Wash hands before entering the work area, when leaving work area, and before handling product.

3. If gloves are worn:

    a. Gloves that handle RTE product must be disposable.

    b. Dispose immediately and replace if anything other than product and food contact surface is touched.

    c. Dispose of gloves when leaving the processing line.

4. Remove outer clothing when leaving RTE areas.

5. Do not wear RTE clothing inside bathrooms or cafeterias.

6. Do not store soiled garments in lockers.

7. Do not eat in the locker room or store food in lockers.

8. Do not store operator hand tools in personal lockers. This equipment must remain in the RTE area at all times.

Management responsibilities should include:

1. Providing hand washing facilities at proper locations.

2. Ensuring the employee receives proper hygiene instruction before starting – use of hand soaps and sanitizers, no-touch dispensing systems, and boot and doorway

sanitizing systems.

3. Developing a system for monitoring employee hygiene practices.

4. Developing a system for tracking the training, tests taken, and certification.

5. Retraining employees before placing back into production.

V. Sanitizers

Cleaning and sanitizing are vital to any effective sanitation program. Through cleaning should be followed by sanitizing. Generally, the cleaning step is to remove all waste materials and soils, and the sanitizing step is to destroy all microorganisms. Careful consideration should be given to selecting both, cleaning and sanitizing solutions. It is important to use solutions that are compatible with the equipment materials, such as stainless steel or heavy plastics, and solutions that are effective in destroying the type of bacteria commonly associated with the type of products you produce. Acidic quaternary ammonia, chlorine dioxide, and peracetic acid compounds were found to be the most effective in destroying attached organisms (Krysinski, L.J., et al;1992).

To aid the cleaning and sanitizing employee in properly selecting and applying the product for its intended application, products that are specifically designed to clean soils in meat and poultry establishments and that are color coded for each application should be selected. An example of this kind of product is Quorum (Ecolab, Inc., St. Paul., MN). Another help for the cleaning employee is to select products with product label and instructions written in English and Spanish.

VI. Sources and Control of Listeria monocytogenes Contamination

Listeria monocytogenes is constantly introduced into the processing environment. It may be introduced in incoming raw product, processing environment or by employees. The following are steps that should be taken to prevent contamination of product with L. monocytogenes after cooking:

1. Verify that cooking or other control measures will eliminate L. monocytogenes. Most meat products implicated in human listeriosis are contaminated with L. monocytogenes after these measures are applied. Undercooked product may introduce L. monocytogenes to food contact surfaces or the environment after cooking and before packaging.

2. Prevent contamination of product contact surfaces and prevent the formation and growth of L. monocytogenes in a niche, especially in areas after the cooking step. A niche is a harborage site within the plant that provides an ideal place for L. monocytogenes to establish and multiply. Certain strains can become established in a processing environment for months or years. L. monocytogenes can be spread from

these sites and re-contaminate food or food contact surfaces between the cooking step and packaging.

Examples of reservoirs and harborages of L. monocytogenes in RTE processing environment
Hollow rollers on conveyors
On-off valves and switches
Worn or cracked rubber seals around doors
Vacuum/air pressure pumps, lines, hoses
Cracked tubular rods on equipment
Air filters
Drains
Condensate from refrigeration unit
Floors
Standing water
Open or gulley drains
Ceilings and over head pipes
Overhead rails and trolleys
Chiller and passageway walls and doors
Chiller shelving
Roller guards
Door handles
Boots
Ice makers
Saturated Insulation
Trolley and forklifts
Compressed air in-line air filters
Trash cans
Cracked hoses
Wet rusting or hollow framework
Walls that are cracked, pitted, or covered with inadequately sealed surface panels
Maintenance and cleaning tools
Space between close fitting metal-to-plastic parts
Space between close fitting metal-to-metal parts

3. Examine routes taken by products from heat treatment, or other control to eliminate L. monocytogenes, to final packaging.

Typical sites of L. monocytogenes contamination
Filling or packaging equipment
Solutions used in chilling food
Peeler, slicer, shredders, blenders, brine chill, casing removal system, scales, or other equipment used after heating and before packaging
Spiral or blast freezers
Conveyors
Bins, tubs, or other containers used to hold food for further processing

4. Frequently clean sites known to support L. monocytogenes using effective cleaning procedures. The following is a recommended frequency for cleaning and sanitizing processing equipment and the plant environment:

    a. Daily

        i. All processing equipment

        ii. Floors and drains

        iii. Waste containers

        iv. Storage areas

    b. Weekly

        i. Walls

    c. Weekly/monthly

        i. Condensate drip

        ii. Coolers

    d. Semiannually

        i. Freezers

5. Maintain equipment and repair parts or machinery in a manner to prevent food deposits that are not easily removed with normal cleaning.

6. Implement a microbial sampling program to monitor and detect sources of L. monocytogenes in the environment. Environmental testing is more effective then product testing alone to monitor and detect Listeria in the environment.

7. Design a sampling scheme to locate a niche before L. monocytogenes becomes established.

    a. Use a statistically designed sampling plans based on probability, or

    b. Use prior experience and familiarity with processing conditions to determine the most likely source of contamination. All processing equipment would sampled but with a bias toward those areas identified as possibly problematic.

    c. Review at least the last month of results to determine trends or to revise sampling scheme.

    d. When a problem area is detected, take corrective action on the affected processing line as opposed to adjacent lines in the area. Target the area corresponding to the line associated with the findings for cleaning. Contamination is usually line specific.

8. Take follow up tests to monitor the area and verify the cleaning results.

Equipment Design

Selecting the appropriate equipment enhances cleaning operations and help control L. monocytogenes in the plant environment. The following are steps to take when selecting equipment:

1. If possible, develop a team (persons from Quality Assurance, Sanitation, Maintenance, and Production) to evaluate equipment before it is purchased or set specific requirements for plant equipment.

2. Have the equipment reviewed by a third-party expert if possible.

3. Select equipment designed to minimize sites on the exterior or interior where L. monocytogenes can grow.

4. Select equipment designed to enhance cleaning.

    a. All areas and parts should be accessible for manual cleaning and inspection or be readily disassembled.

        i. Closed conveyor designs are more difficult to clean. Equipment on the processing line should be as easy to clean as possible.

        ii. Avoid hollow conveyor rollers and hollow framing. If hollow material is used, have a continuous weld seal instead of caulk.

b. Equipment should be self-draining or self-emptying.

5. Select food contact surfaces that are inert, smooth and non-porous.

6. Maintain equipment and machinery by adopting regular maintenance schedules.

    a. Damaged, pitted, corroded, and cracked equipment should be repaired or replaced.

        i. Repair parts or machinery in a manner to prevent food deposits that are not easily removed with normal cleaning.

        ii. Use separate tools for RTE equipment only. Sanitize them before and after each use.

    b. If compressed air is used, maintain and replace in-line filters regularly.

    c. Use lubricants that contain listericidal additives such as sodium benzoate. L. monocytogenes can grow in lubricants that are contaminated with food particles.

    d. Use the appropriate cleaners and sanitizers on surfaces or equipment.

Thoroughly clean and sanitize equipment prior to using in production. Pathogens can live on surfaces that appear visually clean.

VII. Determining the Effectiveness of Sanitation Procedures (Testing for Listeria monocytogenes, Listeria spp. or Listeria-like organisms)

Establishments can verify the effectiveness of their sanitation program by testing food contact surfaces (FCS) and other relevant environmental surfaces. This section includes recommended testing of food contact surfaces for each alternative, a guide to testing for Listeria spp or Listeria-like organisms, and an example of a hold and test scenario.

A. Food Contact Surface and Environmental Testing

The sampling frequencies for FCS testing suggested below should be increased if there is construction, change in the HACCP plan, roof leaks, or other event that could change or increase the probability of product contamination. Samples should be taken at least 3 hours after the start of operation. Up to 5 samples may be composited. However, it is recommended that like surfaces be composited (e.g., food contact surfaces with other food contact surfaces, etc.). The sample locations for the composite sample should be noted to assist in determining the site of contamination. Environmental samples other than food contact surface samples should be taken by the establishment. This will also assist the establishment in locating potential sources of contamination.

1. Alternative 1 – Use of a post-lethality treatment and an antimicrobial agent or process that limits growth of L. monocytogenes.

    i) Conduct tests of food contact surfaces for L. monocytogenes, Listeria spp., or Listeria-like organisms at least twice a year.

    ii) Sample at least 1 square foot area for each surface, if possible.

    iii) Record the test results.

    iv) If test results are positive for L. monocytogenes or Listeria-like or organisms:

        (1) Take corrective action which should include an intensified cleaning and sanitizing.

        (2) Record the corrective actions taken.

        (3) Retest the food contact surface.

        (4) Repeat corrective action and testing until samples are negative for L. monocytogenes or Listeria-like organisms.

        (5) More than 3 consecutive positives should initiate intensified testing.

2. Alternative 2 - Use of a post-lethality treatment or an antimicrobial agent or process that limits growth of L. monocytogenes.

    i) If a post-lethality treatment is used, conduct tests of food contact surfaces for L. monocytogenes, Listeria spp., or Listeria-like organisms at least quarterly.

        (1) Sample at least 1 square foot area for each surface, if possible.

        (2) Record the test results.

        (3) If test results are positive for L. monocytogenes or Listeria-like organisms:

            (a) Take corrective action which should include an intensified cleaning and sanitizing.

            (b) Record the corrective actions taken.

            (c) Retest the food contact surface.

(d) Repeat corrective action and testing until samples are negative for L. monocytogenes or Listeria spp., or Listeria-like organisms.

ii) If an antimicrobial agent is used, conduct tests of food contact surfaces for L. monocytogenes, at least quarterly.

(1) Sample at least 1 square foot area for each surface, if possible.

(2) Record the test results.

(3) If 3 consecutive tests of food contact surfaces are positive for Listeria spp., or Listeria-like organisms:

(a) Take corrective action which should include an intensified cleaning and sanitizing.

(b) Record the corrective actions taken.

(c) Hold the product.

(d) Test product for L. monocytogenes.

(e) Retest the food contact surface.

(f) Repeat corrective action and testing until food contact surface test results are negative for L. monocytogenes, Listeria spp., or Listeria-like organisms.

(g) If the test results for the product are positive for L. monocytogenes,

(i) Recall the product, if necessary, and

(ii) Destroy the product, or

(iii)Re-work the product with a process with a process that is destructive of L. monocytogenes.

3. Alternative 3 – Use of sanitation control measures only to prevent contamination of product with L. monocytogenes.

i) Conduct tests for L. monocytogenes, Listeria spp., or Listeria-like organisms at least four times per month per line for large establishments, two times per month per line for small establishments, and once per month per line for very small establishments. (A large establishment is one that employs more than 500 employees, a small establishment is one that employs from 10 to 499

employees, and a very small establishment is one that employs less than 10 employees and one grossing less than $ 2.5 million in sales.)
FSIS regards production volume as a more important risk factor than establishment size and intends to use volume as one of the primary triggers for when considering its verification activity. For now, regarding deli meat and hotdog operations, FSIS is considering the break-off between high volume and low volume to be approximately 1.3 million pounds yearly, derived from the RTE survey**.**

ii) Sample at least 1 square foot area for each surface, if possible.

iii) Record the test results.

iv) If the first test result of a food contact surface is positive for Listeria spp., Listeria-like organisms, record the corrective actions taken.

v) For establishments producing hotdog or deli meat products, if the second test result of a food contact surface is positive for Listeria spp., Listeria-like organisms:

(1) Take corrective action which should include an intensified cleaning and sanitizing.

(2) Record the corrective actions taken.

(3) Hold the product or recall the product (see hold and test scenario below).

(4) Test for L. monocytogenes at a rate that provides a level of statistical confidence that the product is not adulterated.

(5) Retest the food contact surface each day until the test result is negative for Listeria spp., Listeria-like organisms.

(6) Continue to hold each day's production lot until the test results for the food contact surfaces are negative.

(7) If the test results for the product are positive for L. monocytogenes,

(a) Recall the product, if necessary, and

(b) Destroy the product, or

(c) Re-work the product with a process with a process that is destructive of L. monocytogenes.

vi) For establishments producing products other than hotdogs or deli meats, if 3 consecutive tests of food contact surfaces are positive Listeria spp., or Listeria-like organism:

(a) Take corrective action, which should include an intensified cleaning and sanitizing.

(b) Record the corrective actions taken.

(c) Hold the product.

(d) Test product for L. monocytogenes.

(e) Retest the food contact surface.

(f) Repeat corrective action and testing until food contact surface test results are negative for L. monocytogenes, Listeria spp., or Listeria-like organisms.

(g) If the test results for the product are positive for L. monocytogenes,

(i) Recall the product, if necessary, and

(ii) Destroy the product, or

(iii)Re-work the product with a process with a process that is destructive of L. monocytogenes.

FSIS realizes that some establishments' sanitation and testing program may be exceeding the guidance provided above. In this case, FSIS may put the establishment's product into a lower expected frequency for verification testing within the appropriate sampling frame under the following conditions:

a) The establishment addresses major construction within its control program such that the intensity of sanitation and the verification testing procedures are increased during the time of the disruption and for a period of time following the disruption until the data demonstrate that there is no harborage of L. monocytogenes or its indicator organisms.

b) The establishment has a good history of proper maintenance of the control program, particularly in regards to such things as the sanitation program, reacting to conditions that might indicate that harborage of L. monocytogenes or its indicator organisms is occurring, and appropriately reacting to positive test results for L. monocytogenes or indicator organisms.

B. Guidelines for Listeria spp. and Listeria-like testing for food contact surfaces and other environmental testing

Listeria spp. or Listeria-like organisms are the indicator organisms to be used for L. monocytogenes because their presence indicates the potential presence of the pathogen. If these specific indicator organisms test negative, this is indicative that L. monocytogenes is not present. Aerobic plate counts (APC), total plate counts (TPC), and coliforms are not appropriate indicator tests for L. monocytogenes. Results from these tests do not indicate the presence or absence of the pathogen. However, testing for these organisms can be done in addition to the testing for L. monocytogenes or its indicators to monitor the effectiveness of the cleaning procedures and level of contamination during processing. FSIS microbiology laboratory methods are available and can be downloaded at http://www.fsis.usda.gov/OPHS/microlab/mlgbook.htm

1. Listeria spp. testing

   i) The methodology must employ enrichment prior to Listeria spp. screening.

   ii) Listeria spp. screening is conducted from the enrichment using an immunoassay, nucleic acid assay, or equivalent Listeria spp.-specific technology.

   iii) The above enrichment and screening must be part of a method in use by a government agency (*i.e.*, FSIS or FDA) or validated by a recognized body (*e.g.*, AOAC, AFNOR, ISO, etc.) for the detection of Listeria spp. and/or L. monocytogenes. Specific validation for environmental sampling is encouraged but not a requirement at this time.

2. Listeria-like indicator testing

   i) The methodology must employ enrichment prior to Listeria-like indicator screening.

   ii) The Listeria-like indicator positive screening result may be indicated by the presence of suspect Listeria spp. colonies after selective plating, or may be indicated by biochemical changes to screening broths (*e.g.*, Fraser Broth) that are consistent with the potential presence of Listeria spp.

   iii) The above enrichment and screening must be part of a method in use by a government agency (*i.e.*, FSIS or FDA) or validated by a recognized body (*e.g.*, AOAC, AFNOR, ISO, etc.) for the detection of Listeria spp. and/or L. monocytogenes. Specific validation for environmental sampling is encouraged but not a requirement at this time.

   iv) Aerobic plate counts, ATP assays and other indicator organism tests that do not specifically meet the above requirements may be employed by the

establishment for supplemental sanitation testing. However, these tests do not meet the FSIS expectations for Listeria spp. or Listeria-like indicator food contact and other environmental surface testing programs that may be conducted by the establishment.

C. Hold and Test Scenario

Assuming it takes to 3 days to obtain a test result for Listeria spp., or Listeria-like organisms:

Day 1 – Take food contact surface (FCS) samples

Day 4 – FCS sample positive (from Day 1) for Listeria spp., or Listeria-like organisms.

- ✓ Take Corrective Action
- ✓ Intensified cleaning and sanitizing
- ✓ Test FCS-- target most likely source of contamination, and additional tests in surrounding FCS area
- ✓ Continue production.

Day 7 – Second FCS sample (from Day 4) positive for Listeria spp., or Listeria-like organisms.

- ✓ Take Corrective Action
- ✓ Intensive cleaning and sanitizing
- ✓ Test FCS-- target most likely source of contamination, and additional tests in surrounding FCS area
- ✓ Hold and test product (for L. monocytogenes) for lot implicated in the positive FCS testing.
- ✓ Continue production, hold product from the day's production

Day 8 –

- ✓ Test FCS-- target most likely source of contamination, and additional tests in surrounding FCS area
- ✓ Hold product from this day's production

Day 9 –

- ✓ Test FCS-- target most likely source of contamination, and additional tests in surrounding FCS area
- ✓ Hold product from this day's production

Day 10 –

If FCS sample (day 7 sample) is negative for Listeria spp., or Listeria-like organisms.

- ✓ Continue production and release product from days 7, 8 and 9 production

- ✓ Resume FCS testing according to frequency stated in sanitation program

If FCS sample (day 7 sample) is positive for Listeria spp., or Listeria-like organisms:

- ✓ Hold product from day 10 production.
- ✓ Test product from days 7, 8, 9, and 10 for L. monocytogenes
- ✓ Take corrective action
- ✓ Intensive cleaning and sanitizing
- ✓ Take FCS sample-- target most likely source of contamination, and additional tests in surrounding FCS area

Day 14 – If product is positive for L. monocytogenes, do not release product to commerce and destroy product, or rework product with a process that is destructive of L. monocytogenes.

Every time there is a second or more (consecutive) FCS positive, product is held and tested for L. monocytogenes. Only product lots implicated with a second or more consecutive FCS positive are held and tested. Every time there is a product positive for L. monocytogenes, product is recalled, if not held, and destroyed or reworked with a listericidal process. Once the FCS testing is negative, implying that the corrective action is working, production is continued.

Repeated FCS positives would imply a critical sanitation problem and the establishment need to conduct intensive testing and intensive cleaning and sanitizing. The establishment should have provisions in their FCS testing program for these kinds of situations.

D. Sentinel Site Program Example

Some establishments have adopted a sentinel site program for the control of L. monocytogenes in RTE meat and poultry products. A sentinel site program is similar to traditional Listeria control programs – separate testing programs for the environment and food contact surfaces and increasingly aggressive corrective actions to eliminate Listeria when it is detected. The distinctive characteristic of this control program is that in the case of a positive Listeria test result for a food contact surface area, the sanitation of that particular area will be included in the HACCP plan as a CCP. The CCP is removed when the establishment determines that the food safety hazard has been eliminated and is not reasonably likely to occur.

The CCP is the sanitation program for the particular site and food contact surface sampling as verification of the CCP. If a food contact surface or non-food contact surface tests positive for Listeria spp. or Listeria-like organisms, testing is intensified in the area of the positive.

If a non-food contact surface sampling site is found to be positive for Listeria spp. or Listeria-like organisms during routine monitoring, intensified sampling is initiated as

soon as possible. Under intensified sampling, three samples per day (one each at pre-op, 1st shift, 2nd shift) are analyzed until a total of nine consecutive samples have been taken and are negative for Listeria spp. or Listeria-like organisms at that particular site. Swabs are analyzed for each day of production. If a sample finding is positive, testing of that site continues until nine consecutive samples are negative for Listeria spp. or Listeria-like organisms. Once nine consecutive samples are found negative, that site will returned to routine sampling.

Similarly, the food contact surface site that initially tests positive for Listeria spp. or Listeria-like organisms will be placed under intensified testing. If nine consecutive samples under the intensified testing are negative for Listeria, that site is returned to routine monitoring. However, if the food contact surface tests positive under the initial intensified sampling, sanitation for that area is designated as a CCP since Listeria cannot be considered a hazard not reasonably likely to occur. The site testing positive for Listeria would be considered a suspect harborage for L. monocytogenes and corrective actions taken. Testing becomes the verification step.

Intensified sampling under the CCP requires that 3 samples per day (one each at pre-op, 1st shift, 2nd shift) be taken until nine consecutive samples are negative for **both** Listeria spp. and L. monocytogenes. If a sample is positive for *Listeria* spp. but negative for L. monocytogenes, additional sampling days are added (3 samples per day) until nine consecutive samples are negative for both Listeria spp. and L. monocytogenes. All product that has contact with that particular site must be placed on hold pending testing results.

If nine consecutive samples are negative for Listeria spp. and L. monocytogenes, the site can be returned to routine sampling. Product can be released when the line and production date receive negative test results for L. monocytogenes. Any sites testing positive for L. monocytogenes would require testing of the product.

Sentinel Site Program
Example Flowchart

1. Routine Environmental Sampling
    a. 5 samples/line/week
        i. 3 – food contact surface samples
        ii. 2 – non-food contact surface samples
        iii. Listeria spp.
2. Non-food Contact Surface Testing
    a. If negative for Listeria spp., continue Routine Environmental Testing
    b. If positive for Listeria spp., intensify sampling
        i. Collect 3 samples/site/day for 3 consecutive days for Listeria spp. (9 consecutive samples)
        ii. If 9 consecutive samples are negative for Listeria spp., return to Routine Environmental Sampling
        iii. If any sample is positive, continue sampling 3 samples/site/day until 9 consecutive samples are negative
3. Food Contact Surface (FCS) Testing
    a. If negative for Listeria spp., continue Routine Environmental Testing.
    b. If positive for Listeria spp., intensify sampling.
        i. Collect 3 samples/site/day for 3 consecutive days for Listeria spp. (9 consecutive samples).
        ii. If 9 consecutive samples are negative for Listeria spp., return to Routine Environmental Sampling.
        iii. If any sample is positive, make sanitation for that site a CCP

4. CCP Testing

    a. Collect 3 samples samples/site/day for 3 consecutive days for Listeria spp. **and** L. monocytogenes (9 consecutive samples).

    b. If 9 consecutive samples are negative for Listeria spp. **and** L. monocytogenes, return to Routine Environmental Sampling and eliminate the CCP.

    c. If a sample is positive for Listeria spp. but negative for L. monocytogenes

        i. Place product on hold

        ii. Release product if site and production date have negative results for L. monocytogenes

        iii. Continue testing until 9 consecutive samples are negative for Listeria spp. **and** L. monocytogenes, then return to Routine Environmental Sampling and eliminate the CCP

    d. If any sample is positive for L. monocytogenes, test the product for L. monocytogenes

        i. Reprocess or destroy product testing positive for L. monocytogenes

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